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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The purpose of this study is to determine the functional role of the putative oncogene BORIS in ovarian cancer. The proposed studies will use a newly developed mouse model system to assess the oncogenic potential of BORIS expression in the ovarian surface epithelium, alone and in combination with Rb and p53 knockout. In the past year we have accomplished a number of objectives: i) we have obtained IACUC and Biosafety approvals for the proposed studies, ii) we designed and constructed a new conditional overexpression construct (iZEG-CTCFL) to drive BORIS expression in mice, iii) we have designed a TALEN approach to target iZEG-CTCFL to the ROSA26 locus in mice. The constructs have been injected into fertilized eggs from FVB/N mice, and we are currently awaiting founder mice. iv) we have obtained double conditional knockout mice (Rb, p53) in FVB/N background and bred with FVB/N wild-type mice, obtained F1 and intercrossed to generate stocks of single Rbflox or p53flox mice, v) Ms. Joanna March, the Teal scholar, has learned how to carry out intrabursal injections and has begun these experiments to meet the study objectives, and vi) Ms. March has learned how to isolate and culture OSE cells from mice and has begun to isolate and utilize these cultures to meet the study objectives.

15. SUBJECT TERMS
BORIS, CTCFL, ovarian cancer, mouse models, transgenic mice

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Table of Contents

Page

INTRODUCTION

BORIS (aka CTCFL) is the antagonistic paralog of CTCF, a protein involved in global regulation of chromatin structure and genomic imprinting. In human epithelial ovarian cancer (EOC), BORIS is aberrantly expressed due to promoter DNA hypomethylation. Also, an increased expression ratio of BORIS/CTCF in EOC is associated with global DNA hypomethylation, advanced stage, and reduced survival. These findings suggest BORIS activation is oncogenic in EOC. Based on the known function of CTCF in chromatin insulation, BORIS activation may lead to remodeling of the epigenome, particularly via DNA methylation changes (both hypermethylation and hypomethylation). It is plausible that BORIS activation may act cooperatively with Rb and p53 loss to drive epigenetic changes and EOC progression. BORIS activation in EOC is coincident with activation of E2F target genes, and wildtype p53 is a negative regulator of BORIS expression. To test whether BORIS expression is oncogenic in EOC we have developed a murine transgenic model that allows for the specific expression of BORIS in the ovarian surface epithelium (OSE), following delivery of adenovirus expressing Cre recombinase into the ovarian bursa.

BODY

Task 1. Determine the impact of BORIS expression, alone and in combination with loss of p53 and/or Rb, on EOC development.

1a. IACUC and Biosafety Approvals

The existing IACUC protocol was amended to permit the studies described in the remainder of the proposal and approved in October of 2012. Since this protocol was already in its final year, it was resubmitted and approved for an additional 3 years in October of this year. In addition the Biosafety protocol for the use of adenoviruses was also resubmitted and approved.

1b. Generation of transgenic mouse model with conditional CTCFL (BORIS) transgene inserted at the ROSA26 locus using TALENS in an FVB/N background.

As outlined in the Statement of Work, we decided to generate a new *CTCFL* (*BORIS*) transgenic mouse so that it would be in the FVB/N background. In addition, we decided to remake the transgene in the iZEG conditional expression construct instead of the originally used pCLEG vector (described in original proposal) because of it contains reporters for both pre-activation (LacZ) and post-activation (EGFP) states. Finally, to help prevent epigenetic gene silencing that can take place with randomly inserted transgenes, we are using newly-developed transcription activator-like effector nucleases (TALENs) designed to target the ROSA26 locus. Construction of the new transgene (iZEG-CTCFL) has been completed (Fig. 1A) and the construct injected, along with ROSA26 TALENS, into fertilized eggs from FVB/N mice. We are currently waiting for founder mice which will be crossed with mice carrying floxed p53 and pRb alleles to generate the iZEG-CTCFL containing strains described in the SOW. Intrabursal injection of adeno-Cre will activate BORIS expression by mediating excision of the LacZ-neo-polyA csassette (Fig. 1B).

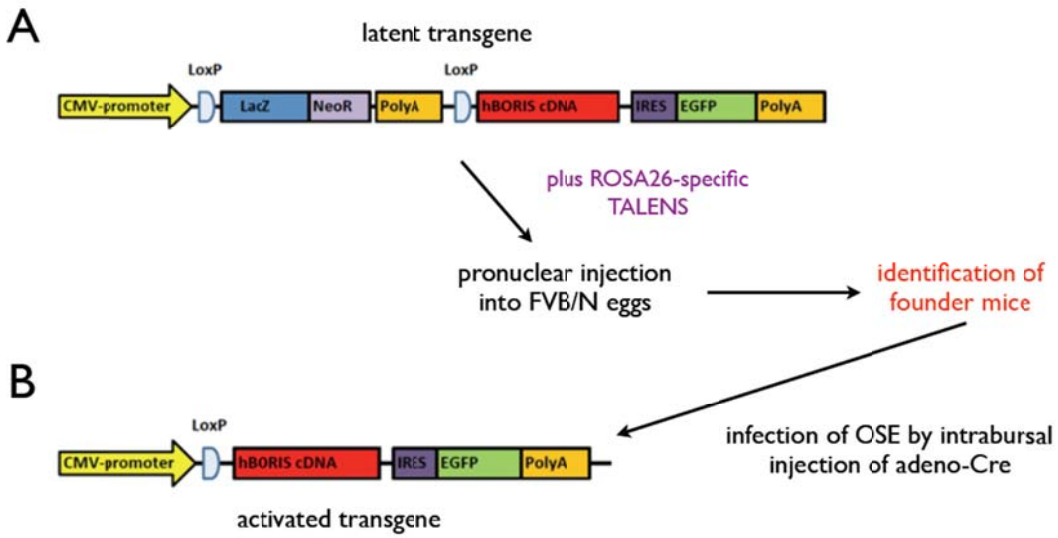

Figure 1. Construction of a new BORIS transgene

1c. Generation of experimental mice and intrabursal injection of adenovirus expressing Cre-recombinase

The initial double conditional knockout mouse ($pRb^{flox/flox}$;$p53^{flox/flox}$ in FVB/N background) has been bred with FVB/N wild type mice, and F1 mice intercrossed to generate stocks of single $pRb^{flox/flox}$ and $p53^{flox/flox}$ knock out mice. Ms. Joanna March, the recipient of the Teal Fellowship, is carrying out ovarian intrabursal injections using these mice after receiving appropriate training from Dr. Sandra Buitrago, the RPCI veterinarian, as well as from Dr. Andrea Flesken-Nikitin at Cornell University, the first to describe this technique. She has injected the first set of $pRb^{flox/flox}$;$p53^{flox/flox}$ and $p53^{flox/flox}$ females with adeno-Cre-EGFP and is monitoring them for infection efficiency and tumor formation.

Task 2. Determine the impact of BORIS expression, alone and in combination with loss of Rb and/or p53, on epigenomic and genomic stability in EOC.

Work on Task 2 has not yet begun. Once BORIS transgenic mice are generated, and tumor tissues are harvested from these, we can begin work on Task 2.

Task 3. Determine the impact of BORIS expression, alone and in combination with loss of p53 and/or Rb, on OSE transformation *in vitro*.

While visiting Dr. Flesken-Nikitin at Cornell, Ms. March also learned to isolate and culture ovarian surface epithelial (OSE) cells. She has successfully established OSE cultures from double conditional knockout mice ($pRb^{flox/flox}$;$p53^{flox/flox}$) as well as from $p53^{flox/flox}$ knock-out mice. Following, infection with adeno-Cre virus, these cells will be tested for growth in soft agar and invasive growth through Matrigel.

KEY RESEARCH ACCOMPLISHMENTS

- Obtained IACUC and Biosafety approvals for the proposed studies.
- Designed and constructed a new conditional overexpression construct (iZEG-CTCFL) to drive BORIS expression in mice.
- Designed a TALEN approach to target iZEG-CTCFL to the ROSA26 locus in mice. The constructs have been injected into fertilized eggs from FVB/N mice, and we are currently awaiting founder mice.
- Obtained double conditional knockout mice (Rb, p53) in FVB/N background and bred with FVB/N wild-type mice, obtained F1 mice and intercrossed to generate stocks of single Rbflox or p53flox mice.
- Ms. Joanna March, the Teal scholar, learned how to carry out intrabursal injections and has begun these experiments to meet the study objectives.
- Ms. March learned how to isolate and culture OSE cells from mice and has begun to isolate and utilize these cultures to meet the study objectives.

REPORTABLE OUTCOMES

N/A

CONCLUSION

We have made significant progress towards functionally assessing the role of BORIS in ovarian cancer. We have completed the necessary experimental design, have trained with the necessary experimental methods, and in the process of producing mice to test the central hypotheses of the proposal. Successful completion of this proposal will reveal important new information about the molecular pathology of ovarian cancer, and the role of aberrant BORIS expression in the disease. This knowledge can be used to develop novel biomarkers and therapeutic approaches for ovarian cancer.

REFERENCES

N/A

APPENDICES

N/A

SUPPORTING DATA

N/A